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Molecular and physiological comparison of *Azospirillum* spp. isolated from *Rhizoctonia solani* mycelia, wheat rhizosphere, and human skin wounds

Michael F. Cohen, Xiang Y. Han, and Mark Mazzola

Abstract: Four phenotypically similar bacterial strains isolated from fungal, plant, and human sources were identified as *Azospirillum* species. Strains RC1 and LOD4 were isolated from the mycelium of the apple root pathogen *Rhizoctonia solani* AG 5 and from the rhizosphere of wheat grown in apple orchard soil, respectively. Strains C610 and F4626 isolated from human wounds were previously misclassified as *Roseomonas* genomospecies 3 and 6. All four strains demonstrated close similarities in 16S rRNA gene sequences, having ≥97% identity to *Azospirillum brasilense* type strain ATCC 29145 and <90% identity to *Roseomonas gilardii*, the *Roseomonas* type strain. Extensive phenotypic similarities among the four strains included the ability of free-living cells to fix N_2. Cells of strains RC1, LOD4, and C610 but not of strain F4626 could be induced to flocculate by incubation with 10 mmol·L^{-1} glycerol or fructose in medium containing 0.5 mmol·L^{-1} NO_3^-. Our results indicate a wide range of potential sources for *Azospirillum* spp. with the isolation of *Azospirillum* spp. from human wounds warranting further investigation.

Key words: Azospirillum brasilense, *Roseomonas fauriae*, flocculation, *Rhizoctonia solani*.

Résumé : Quatre souches de bactéries phénotypiquement semblables ont été isolées à partir de sources fongiques, végétales et humaines, et identifiées comme étant des espèces de *Azospirillum*. Les souches RC1 et LOD4 ont été isolées respectivement du mycélium de *Rhizoctonia solani* AG 5, un pathogène des racines du pommier, et de la rhizosphère de blé cultivé dans de la terre de verger. Les souches C610 et F4626 ont été isolées de blessures humaines et avaient auparavant été classifiées incorrectement en tant qu'espèces génomiques 3 et 6 de *Roseomonas*. Les séquences de l'ARNr 16S des quatre souches étaient fortement semblables à celle de la souches type de *Azospirillum brasilense* ATCC 29145, avec ≥97 % d'identité, et avaient <90 % d'identité avec *Roseomonas gilardii*, la souches type de *Roseomonas*. La capacité des cellules libres à fixer le N_2 faisait partie des multiples similarités phénotypiques entre les quatre souches. Un incubation des cellules des souches RC1, LOD4 et C610, mais non de F4626, avec 10 mmol·L^{-1} de glycérol ou du fructose dans un milieu contenant 0,5 mmol·L^{-1} NO_3^- a provoqué la floculation de celles-ci. Nos résultats révèlent l'existence d'une grande variété de sources potentielles d'*Azospirillum* spp. En particulier, l'isolation d'*Azospirillum* spp. à partir de blessure humaines sollicite un examen plus approfondi.

Mots clés : Azospirillum brasilense, *Roseomonas fauriae*, floculation, *Rhizoctonia solani*.

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Azospirillum is a genus of plant-growth-promoting bacteria commonly recovered from soil and from associations with plant roots, especially grasses, both in the rhizosphere and as endophytes (Steenhoudt and Vanderleyden 2000). The capacity of *Azospirillum* spp. cells to flocculate is dependent on the formation of extracellular polysaccharides and is positively correlated with root colonization efficiency (Pereg-Gerk et al. 1998) and adhesion to arbuscular mycorrhizal fungal structures (Bianciotto et al. 2001).

Azospirillum spp. are diazotrophic (Tarrand et al. 1978), with many strains accumulating pink carotenoids thought to protect nitrogenase from oxidative damage (Nur et al. 1981). The reported phenotypic characteristics of certain pink-pigmented oxidative bacteria isolated from human infections match those of *Azospirillum* spp. This similarity, however, was not noted by the authors of the original reports (Wallace

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Table 1. Percentage of selected cellular fatty acids from the strains examined in this study.

	Organism					
Fatty acid[a]	*Azospirillum brasilense* 29145	*Azospirillum* sp. RC1	*Azospirillum* sp. LOD4	*Roseomonas fauriae*	*Roseomonas* genomospecies 6	*Roseomonas gilardii*
16:0	6.3%	6.6%	6.3%	5.0%	6.1%	17.5%
16:0 3OH	4.4%	3.9%	4.6%	4.2%	4.2%	7.0%
18:1[b]	63.4%	64.1%	66.0%	59.3%	59.1%	53.5%
19:0 CYCLO ω 8*c*	0.0%	0.0%	0.0%	0.0%	0.0%	9.3%
Similarity index[c]	0.87%	0.90%	0.80%	0.67%	0.73%	0.00%

Note: Reported results are the means of at least three independent measurements.

[a]Fatty acids are designated as X:Y ωZ, where X is the number of carbon atoms, Y the number of double bonds, and Z the position of the ultimate double bond from the terminal methyl group; c, *cis*, t, *trans*.

[b]Includes 18:1 isoforms ω7*c*, ω9*t*, and ω12*t*.

[c]Microbial Identification System Similarity Index relative to the *A. brasilense* profile in the MIDI version 3.9 library (highest value reported).

et al. 1990; Rihs et al. 1993). Due mainly to their pink color and clinical sources, these bacteria were classified into genomospecies 3 and 6 of the newly described genus *Roseomonas*, in spite of their low DNA relatedness to other *Roseomonas* genomospecies (7%–17% total DNA cross hybridization at 70 °C).

Here, we report the isolation of *Azospirillum* spp. strains from the rhizosphere of wheat and from the fungus *Rhizoctonia solani* AG 5, an etiologic agent of apple replant disease (Mazzola 1997, 1998). We present comparative analyses among *Azospirillum* spp. strains and *Roseomonas* genomospecies clinical isolates that demonstrate a close relationship between *Azospirillum* spp. and *Roseomonas* genomospecies 3 (*Roseomonas fauriae*) and 6.

Since its isolation from the roots of an apple tree (*Malus domestica* Brokh. 'Gala/M26') in 1995 (Moxee, Wash.) (Mazzola 1997) *Rhizoctonia solani* AG strain 5-103 has been maintained in our laboratory on 1/5 strength potato dextrose agar (Difco Laboratories, Detroit, Mich.) amended with 100 $\mu g \cdot mL^{-1}$ of ampicillin. Bacterial strain RC1 was isolated in November 2002 by plating a distilled water rinse of *Rhizoctonia solani* AG strain 5-103 onto 1/10 strength tryptic soy agar (Difco) containing precipitated calcium oxalate, prepared as described by Jayasuriya (1955). Strain LOD4 was isolated by enriching for oxalate-degrading bacteria from the rhizosphere of wheat *Triticum aestivum* L. 'Lewjain' cultivated in pots using the method of Jayasuriya (1955). Prior to isolation of strain LOD4, the Wenatchee Valley College – Auvil orchard soil (Mazzola 1998) contained in the pots had been cropped to four successive 28-day cycles of this wheat cultivar. Strain LOD4 was derived from a colony that induced zones of clearing on agar medium containing calcium oxalate. *Roseomonas gilardii* ATCC 49956 (the *Roseomonas* type strain), *Roseomonas fauriae* C610 (ATCC 49958, the type strain for genomospecies 3), and *Roseomonas* genomospecies 6 strain F4626 (ATCC 49961, the only known member of this genomospecies), originally isolated from water, a human hand wound, and a breast incision, respectively (Rihs et al. 1993), were kindly provided by P. Levett of the Centers for Disease Control and Prevention, Atlanta, Ga.

To determine the capacity for bacterial growth on various C and N sources, a mineral salts medium was designed having 15 $mmol \cdot L^{-1}$ K_2HPO_4, 10 $mmol \cdot L^{-1}$ NaH_2PO_4, 6.8 $\mu mol \cdot L^{-1}$ $CaCl_2$, 1 $mL \cdot L^{-1}$ of microelements solution (Jayasuriya 1955), and 0.8 $mmol \cdot L^{-1}$ $MgSO_4$ (added after autoclaving). N was added as 5 $mmol \cdot L^{-1}$ $(NH_4)_2SO_4$ (+N medium) or as 0.5 $mmol \cdot L^{-1}$ KNO_3 (low N medium) or was excluded (–N medium). C-source stock solutions (1 $mol \cdot L^{-1}$) were autoclaved separately and added to achieve 10–50 $mmol \cdot L^{-1}$ final concentrations. For growth rate determinations, bacteria were cultured at 30, 32, 35, 37, and 40 °C in tryptone – yeast extract (TYE) medium containing 10 $g \cdot L^{-1}$ of tryptone and 5 $g \cdot L^{-1}$ of yeast extract (Difco). Increases in cell numbers were monitored by measuring the optical density at 600 nm (OD_{600}) of samples periodically removed from the cultures.

Sequencing of 16S rRNA genes was carried out as described previously (Han et al. 2002). Sequence comparisons were made using Nucleotide BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and ClustalW (http://www.ebi.ac.uk/clustalw). The sequences of PCR primers, forward (5′-ACATCGCCAACGACGTCAT-3′) and reverse (5′-ATACGGACCCGAGTAATCCCA-3′), were chosen from a 773-bp region internal to the *nifD* nitrogenous subunit gene of *Azospirillum brasilense* ATCC 29145 (Passaglia et al. 1991). PCR reaction conditions were as previously described (Gu and Mazzola 2003), except for annealing at 60 °C for 40 s and extension at 72 °C for 90 s. Genomic DNA from *Pseudomonas fluorescens* Q2-87 was utilized as a negative control. PCR products were cloned into pCR4-TOPO (Invitrogen, Carlsbad, Calif.) and sequenced on an ABI 377 automated sequencer, using the dye terminator cycle sequencing ready reaction kit (PE BIOsystems, Warrington, England). *Roseomonas gilardii* DNA from a laboratory culture and our source stock were amplified, cloned, and sequenced as above or were sequenced independently by SeqWright (Houston, Tex.), respectively.

Bacteria were subjected to analysis of fatty acid methyl esters, using a Microbial Identification System equipped with an HP5890 series gas chromatograph, HP 3365 Chem Station, and version 3.9 of the aerobe library (Microbial ID, Inc., Newark, Del.), following the manufacturer's recommended procedures. Similarity indices = 0.5 indicate a good match (MIDI Microbial Identification System 1993). C-source oxidation profiles were determined by automated

Table 2. 16S rRNA sequence comparisons.

	Percent identity of aligned sequence					
	Azospirillum sp. LOD4[a]	*Azospirillum brasilense* ATCC 29145	*Azospirillum* sp. 129/1	*Roseomonas* genomospecies 6	*Roseomonas fauriae*	*Roseomonas gilardii*
Azospirillum sp. LOD4[a]	100 (1470/1470)					
Azospirillum brasilense ATCC 29145	97.1 (1373/1414)	100 (1442/1442)				
Azospirillum sp. 129/1	97.1 (1349/1389)	97.7 (1330/1362)	100 (1384/1384)			
Roseomonas genomospecies 6	97.2 (1405/1446)	97.6 (1404/1439)	97.2 (1339/1378)	100 (1483/1483)		
Roseomonas fauriae	97.0 (1411/1454)	99.4 (1430/1439)	97.7 (1346/1378)	97.9 (1450/1481)	100 (1489/1489)	
Roseomonas gilardii	88.3 (1191/1349)	88.3 (1194/1352)	88.7 (1097/1237)	89.9 (1205/1340)	88.6 (1236/1395)	100 (1482/1482)

Note: Ratios in parentheses indicate the number of aligned bases to total aligned bases. GenBank accession Nos.: *Azospirillum* sp. LOD4 (AY283791); *A. brasilense* ATCC 29145 (AY324110); *Azospirillum* sp. 129/1 (X79726) (Fani et al. 1995); *Roseomonas* genomospecies 6 (AY150050) (Han and Levett); *Roseomonas fauriae* (AY150046) (Han and Levett), which is 99.4% identical to AF533354 (Bernard et al.); and *Roseomonas gilardii* (AY150045) (Han et al. 2003).

[a]Identical to strain RC1 rDNA within a sequenced 575-bp region.

reading of inoculated GN2 MicroPlates™ using the MicroLog™ System 4.0 (Biolog Inc., Hayward, Calif., USA), following 16–24 h of incubation at 35 °C.

Gelatin-hydrolysis was assayed in TYE medium containing 4% gelatin. Esculin hydrolysis was assayed on TYE agar slants containing 1 $g \cdot L^{-1}$ of esculin and 0.5 $g \cdot L^{-1}$ of ferric ammonium citrate. The concentration of nitrite in stationary-phase nitrate broth (Difco) cultures was determined colorimetrically (Sakihama et al. 2002). Carotenoids were extracted from cells with acetone–methanol (7:2, *v*/*v*), according to the method of Nur et al. (1981). For acetylene reduction assays, colonies were scraped from the surface of 30 $mmol \cdot L^{-1}$ malate–N agar medium (or +N medium for *Roseomonas gilardii*) and suspended in 5 mL of 30 $mmol \cdot L^{-1}$ malate–N liquid medium in 1.5 cm × 15 cm test tubes. The tubes were capped with serum stoppers and injected with 1.25 mL of acetylene gas. After 15 h of incubation at 35 °C the formation of ethylene was determined by injecting 0.5 mL of the gas phase into a Hewlett Packard 5880 gas chromatograph equipped with a 15 m Restek RTX-UPLOT N column heated to 50 °C. Units of acetylene reduction activity are reported as nanomoles of ethylene formed per milligram of cell (dry weight).

Flocculation experiments were conducted based on a previously published procedure (Sadasivan and Neyra 1985). Cells from exponential-phase TYE-grown cultures were harvested by centrifugation at 4000*g* for 10 min at 4 °C and washed in twice the original volume with 100 $mmol \cdot L^{-1}$ phosphate buffer (pH 6.8). Following a second centrifugation, cells were resuspended to an OD_{600} of 0.38 in 25 mL of low-N medium containing 10 $mmol \cdot L^{-1}$ C source or no C as a control. The cell suspension was then shaken at 150 r/min in 125-mL Erlenmeyer flasks at 35 °C for 16 h, and the flocs were quantified by filtering through a Whatman No. 1 filter paper, as previously described (Sadasivan and Neyra 1985).

Characterization of isolates derived from apple orchard

Fatty acid methyl ester microbial identification system analysis indicated a good match of strain RC1 with *A. brasilense* (Table 1). We compared strain RC1 to another pink, oxalate-degrading isolate, designated strain LOD4, recovered from the rhizosphere of wheat grown in soil derived from apple orchard. As with strain RC1, strain LOD4 had a fatty acid profile closest to that of *A. brasilense* (Table 1).

For database comparisons, the 16S rRNA genes of strain LOD4 and *A. brasilense* ATCC 29145 (the type strain) were sequenced to 1470 and 1442 bp, respectively; a 575-bp region of strain RC1 16S rDNA was sequenced and found to be identical to the corresponding region of strain LOD4 16sDNA. Among published *Azospirillum* spp. sequences, strain LOD4 16S rDNA was found to have highest identity (97.1%) with the 16S rDNA of *A. brasilense* ATCC 29145 and *Azospirillum* sp. 129/1 (Table 2), which clusters phenotypically and genetically with *A. brasilense* (Fani et al. 1995), and had less than 96% identity with sequences from other *Azospirillum* spp.

Cells from overnight cultures of strains RC1 and LOD4 were Gram negative, catalase positive, motile, and had a semi-helical shape, characteristic of *Azospirillum* spp. Growth rates were optimal at 37 °C, with mean generation times of

Table 3. Carbon source utilization by the strains examined in this study.

Substrate	*Azospirillum brasilense* 29145	*Azospirillum* sp. RC1	*Azospirillum* sp. LOD4	*Roseomonas fauriae*	*Roseomonas* genomospecies 6	*Roseomonas gilardii*
Oxidation of:						
L-Arabinose	+	+	+	+	–	+
D-Arabitol	–	–	–	–	–	+
D-Galactose	–	–	–	–	–	+
D-Mannitol	–	–	–	–	–	+
D-Sorbitol	–	–	–	–	–	+
D-Galacturonic acid	+	+	+	–	–	+
D-Gluconic acid	+	w	+	+	+	–
γ-Hydroxybutyric acid	–	–	–	–	–	+
Glycyl-L-aspartic acid	–	–	–	–	–	+
Glycyl-L-glutamic acid	–	–	–	–	–	+
L-Phenylalanine	–	–	–	–	–	+
L-Pyroglutamic acid	+	+	+	+	+	–
L-Serine	–	–	–	–	–	+
γ-Amino butyric acid	+	+	+	+	+	–

Note: Substrates listed are those that were differentially oxidized by the strains on Biolog® GN2 plates incubated for 16–24 h and gave consistent results over three independent trials. w, weak positive reaction; +, positive reaction; –, negative reaction.

Fig. 1. Multiple alignment of a region within *Azospirillum* spp. 16S rRNA gene sequences grouped on the basis of the presence or absence of a 15-bp gap. GenBank accession Nos. are as in Table 2 with the addition of *Azospirillum brasilense* DSM 1859 (X79733), *A. lipoferum* F (X79736) (Fani et al. 1995), *A. doebereinerae* (AJ238567), *A. irakense* KBC1 (Z29583), *A. halopraeferens* DSM 3675 (Z29618), *A. brasilense* SpF94 (X79740), *A. brasilense* ATCC 29145 (AY324110), and *A. lipoferum* WO3 (X79741).

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Azospirillum sp. 129/1          GCTAACACCGGATGTGCCCTTC---------------GGGG-AAAG 151
A. brasilense DSM 1859          GCTAACACCGGATACGTCCTTC---------------GGGGGAAAG 151
Azospirillum sp. LOD4           GCTAACACCGGATGTGCCCTTC---------------GGGGGAAAG 173
A. lipoferum F                  GCTAACACCGGATGTGCCCTTC---------------GGGG-AAAG 151
A. doebereinerae                GCTAACACCGGATACGCCCTTC---------------GGGGGAAAG 138
A. irakense                     GCTAATACCGGATGTCCCCTGC---------------GGGGGAAAG 134
A. halopraeferens               GCTAACACCGGATACGCCCTTC---------------GGGGGAAAG 133

A. brasilense SpF94             GCTAACACCGGATACGTCCCCCAGAGAGATTTNGGCCGGAG-AAAG 166
A. brasilense ATCC 29145        GCTAACACCGGATACGTCCCTCAGAGAGATTTGGGC-GGAG-AAAG 165
Roseomonas genomospecies 6      GCTAACACCGGATACGCCCCCCAGACAGATTTGGGC-GGAG-AAAG 177
R. fauriae                      GCTAACACCGGATACGTCCCCCAGAGAGATTTGGGC-GGAG-AAAG 183
A. lipoferum WO3                GCTAACACCGGATACGTCCCCCAGAGGAAATGTGGGCGGAG-AAAG 161
                                ***** *******  * **  *                ** * ****
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1.0 and 0.94 h^{-1} for strain RC1 (n = 3) and strain LOD4 (n = 5), respectively. Of the seven described species of *Azospirillum*, only *A. brasilense* and *A. lipoferum* have a 37 °C growth rate optimum (Eckert et al. 2001). Unlike *A. lipoferum*, but like *A. brasilense*, neither strain LOD4 nor RC1 required biotin for growth on defined medium.

Biolog GN2 MicroPlates™ inoculated with *A. brasilense* ATCC 29145 strains RC1 or LOD4 revealed identical carbon substrate oxidation profiles except for poor utilization of D-gluconic acid by strain RC1 (Table 3); the 24-h-growth yield of strain RC1 on +N medium with 30 $mmol \cdot L^{-1}$ gluconic acid was 20-fold lower than that of strain LOD4 (data not shown). The MicroLog™ System 4.0 MicroPlate reader, which does not include *Azospirillum* spp. within its species library, indicated matches of *A. brasilense* ATCC 29145 as well as strains RC1 and LOD4 with *Roseomonas fauriae*, a finding that warranted investigation of *Roseomonas* taxonomy in relation to *Azospirillum* spp.

Comparisons with *Roseomonas* genomospecies

GenBank-deposited 16S rDNA sequences from *R. fauriae* and *Roseomonas* genomospecies 6 were found to have close identity (up to 99.4%) with *Azospirillum* spp. sequences but not with other *Roseomonas* genomospecies (Table 2) (Han et al. 2003). A 15-bp sequence, found in some strains of *A. lipoferum* and *A. brasilense* (including ATCC 29145), was present in the 16S rRNA gene of *Roseomonas fauriae* and *Roseomonas* genomospecies 6 (Fig. 1).

Fatty acid profiles of *Roseomonas fauriae* and *Roseomonas* genomospecies 6 also display a closer affinity with *A. brasilense* than with *Roseomonas* genomospecies (Table 1). Previous fatty acid methyl ester analysis of pink-pigmented oxidative bacteria had indicated that group IV organisms (later classified as *Roseomonas fauriae*) were not closely related to those bacteria that were later classified into *Roseomonas* genomospecies 1, 2, 4, and 5 (Wallace et al. 1990). In fact, although it was not noted at the time, the

group IV fatty acid profile was quite similar to that of the *Azospirillum* lipoferum–brasilense group profile previously published by Schenk and Werner (1988).

The ability of free-living cells to fix N_2, characteristic of the genus *Azospirillum*, was apparent in strains RC1, LOD4, *Roseomonas fauriae*, and *Roseomonas* genomospecies 6; based on their acetylene reduction activities of 9.3, 8.6, 9.0, and 12.4 U, respectively, their growth on organic acids without a source of combined N, and the presence of *nifD*-specific PCR amplification products (Fig. 2). The genus *Roseomonas* belongs to the family *Methylobacteriaceae*, which contains at least one species, *Methylobacterium nodulans*, which can reportedly fix N_2 in nodules of *Crotalaria* spp. (Sy et al. 2001). *Roseomonas gilardii* (genomospecies 1) did give a *nifD*-specific PCR product (Fig. 2) but required combined N for growth and did not reduce acetylene under our assay conditions. The sequence of the *nifD*-specific PCR products from strain LOD4, *Roseomonas fauriae*, *Roseomonas* genomospecies 6, and *Roseomonas gilardii* (Accession Nos. AY42869–AY42872) showed close similarity to published *nifD* sequences from *A. brasilense* ATCC 29145 (M64344) and a *Burkholderia* sp. (AF194084) (data not shown).

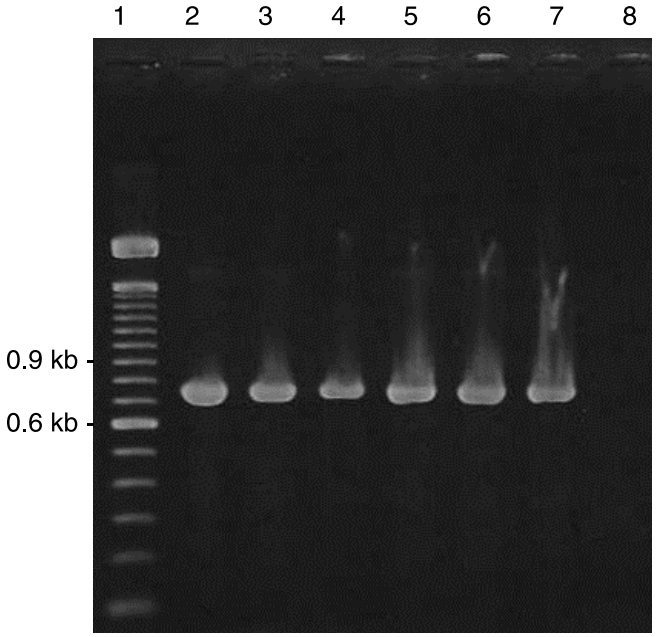
The C-substrate oxidation profiles of *Roseomonas fauriae*, and *Roseomonas* genomospecies 6 were much more like those of *Azospirillum* spp. than that of *Roseomonas gilardii* (Table 3). Likewise, *Roseomonas fauriae* and *Roseomonas* genomospecies 6 but not *Roseomonas gilardii* hydrolyzed esculin and reduced nitrate to nitrite; of the six *Roseomonas* genomospecies characterized by Rihs et al. (1993), only isolates of *Roseomonas fauriae* and *Roseomonas* genomospecies 6 were capable of nitrate reduction. The lower absorption peak wavelength of *Roseomonas gilardii* cellular methanol–acetone extracts (479 nm) compared with that of strains RC1, LOD4, *Roseomonas fauriae*, and *Roseomonas* genomospecies 6 (489–492 nm) was manifest as a noticeably different pink colony color. Additionally, the growth rate of *Roseomonas gilardii* was significantly slower at 35 °C than that of the other four strains; 0.70 ± 0.10 h^{-1} versus 0.95 ± 0.10 h^{-1}(± range, $n = 2$ per strain), respectively.

Overnight incubation in mineral medium containing fructose and a low concentration of combined N has been found to induce flocculation in all *Azospirillum* spp. strains for which this trait has been examined (Sadasivan and Neyra 1985; Burdman et al. 1998). We found that strains RC1 and LOD4 and *Roseomonas fauriae* exhibited flocculation under these conditions and also in medium supplemented with glycerol in place of fructose (Fig. 3). Accumulation of brown-pigmented melanin-like granules was apparent inside the flocs of all three strains (Fig. 3B), consistent with previous reports on *A. brasilense* flocculation (Sadasivan and Neyra 1987; Pereg-Gerk et al. 1998). Interestingly, although *Roseomonas* genomospecies 6 shares many similarities with *Azospirillum* spp., it did not flocculate under inducing conditions (Fig. 3A), instead cells remained motile. Another distinctive feature of *Roseomonas* genomospecies 6, compared with other organisms in this study, was its sensitivity to gelatin, a common trait among bacteria (Reimer et al. 1987).

Taxonomic implications

Strains RC1 and LOD4 were both isolated from association with organisms in soils taken from Central Washington apple orchards that are only 100 km apart. Based on their genotypic and phenotypic profiles they appear to be most closely related to *A. brasilense*. Exudation of organic acids by grass roots (Vancura 1964) and fungal mycelia (Yang et al. 1993) present in the apple orchard undergrowth may support associated *Azospirillum* spp. Strain RC1 is most likely retained by *Rhizoctonia solani* AG 5 during the course of root infection, since bacteria matching the metabolic profile of strain RC1 were recovered from mycelia isolated from infected apple or wheat roots 4 weeks after inoculation (data not shown).

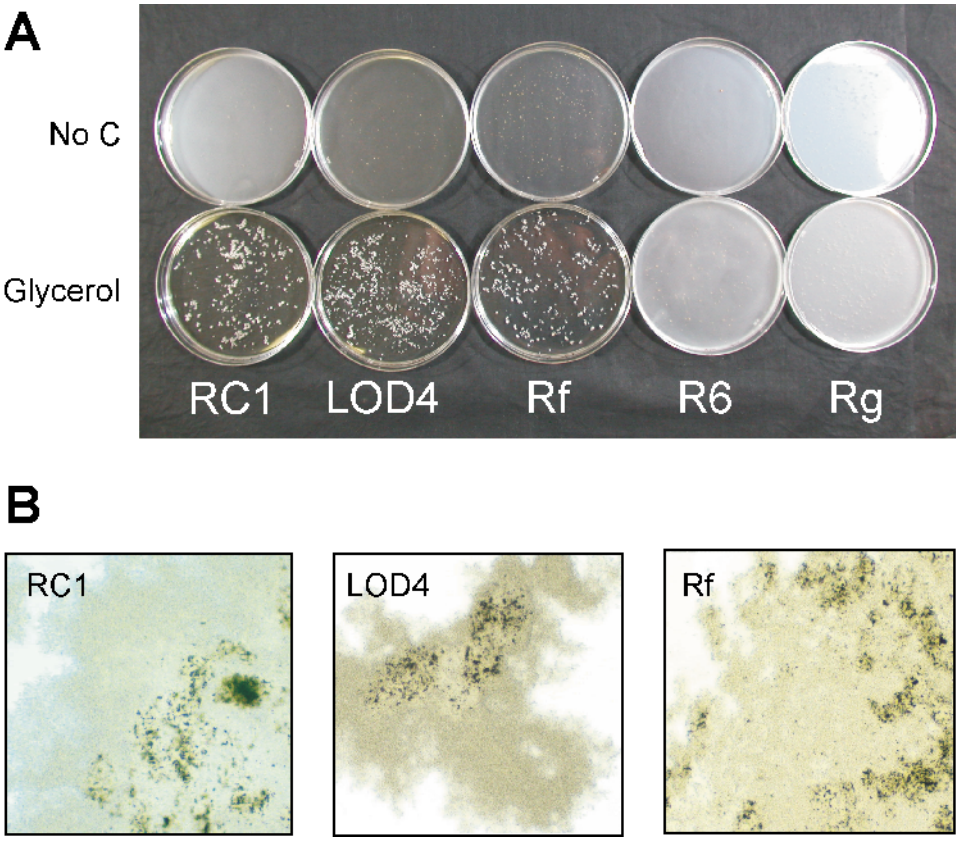
Fig. 2. PCR-amplified products from *nifD*-specific primers. For lanes 2–8, each well was loaded with an 8-μL volume of reaction product. Lane 1, 1 μg of 100-bp marker (Invitrogen, Carlsbad, Calif.); lane 2, *Azospirillum brasilense* ATCC 29145; lane 3, *Azospirillum* sp. RC1; lane 4, *Azospirillum* sp. LOD4; lane 5, *Roseomonas fauriae*; lane 6, *Roseomonas* genomospecies 6; lane 7, *Roseomonas gilardii*; lane 8, *Pseudomonas fluorescens* Q2-87.



In the article by Rihs et al. (1993) that proposed the genus *Roseomonas*, DNA hybridizations showed a maximum of only 15% and 17% relatedness of *Roseomonas* genomospeices 3 and 6, respectively, with members of other *Roseomonas* genomospecies. In addition, the published phenotypes of all characterized *Roseomonas* genomospecies 3 strains and the single known genomospecies 6 strain are more similar to those of *Azospirillum* spp. than to other *Roseomonas* genomospecies. Our results, combined with those of previous studies (Schenk and Werner 1988; Wallace et al. 1990; Rihs et al. 1993), indicate that establishment of *Roseomonas* genomospecies 3 and 6 was not justified and that isolates designated as such should be reclassified into the genus *Azospirillum*.

Further research should be conducted to determine if these *Azospirillum* spp. strains can cause disease in humans, especially since *Azosprillum* spp. are commercially available in some countries as plant-growth-promoting inoculants

Fig. 3. (A) Response to flocculation induction by overnight incubation in low-N medium containing 10 mmol·L^{-1} glycerol (bottom) or no C source (top). (B) Light micrographs of flocs sampled from flocculation-inducible strains; magnification, × 140. Abbreviations: RC1, *Azospirillum* sp. RC1; LOD4, *Azospirillum* sp. LOD4; Rf, *Roseomonas fauriae*; R6, *Roseomonas* genomospecies 6; Rg, *Roseomonas gilardii*.



(Dobbelaere et al. 2001). The discovery that some *Burkholderia* spp. strains have the potential to act as human pathogens has spurred the development of more systematic methods for classification within the genus as well as improved risk assessment of those strains used as environmental inoculants (Parke and Gurian-Sherman 2001; Coenye and Vandamme 2003).

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